DIETARY FACTORS AFFECTING RAT SERUM LIPASE.

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DIETARY FACTORS AFFECTING RAT SERUM LIPASE.

A DISSERTATION

SUBMITTED TO THE SCHOOL OF GRADUATE STUDIES

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF MASTER OF SCIENCE.

FACULTY OF ARTS AND SCIENCE.

DEPARTMENT OF BIOCHEMISTRY.

by

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ABSTRACT.

The effects of various dietary factors on rat serum lipase were tested and from the experimental data obtained the following conclusions were made: Variations in serum tributyrinase levels of normal adult and weanling male rats could not be accounted for on the basis of consumption of Purina Fox Checkers. The high tributyrinase levels of alloxan diabetic adult male rats can be correlated to the amount of Checkers ingested daily.

It has been shown that the serum tributyrinase levels of adult male rats are elevated with increased ingestion of dietary Crisco, butter, cottonseed oil, linseed oil, corn oil, and olive oil. There were significant differences in the activity of the enzyme which depended on the nature of the dietary fat. On the other hand, the serum tributyrinase levels of weanling male rats decreased with increased ingestion of Crisco and this may be accounted for on the basis of diminished protein consumption.

The lowest tributyrinase levels were obtained with diets free of fat. These values were much lower than those obtained with fasting rats. The ingestion of fat soluble vitamins A, D, and E, or glycerol, does not affect these basal enzyme levels. Ingestion of stearic or oleic acid increased the lipolytic activity of rat serum.

From inanition experiments it was possible to conclude that a tributyrin hydrolyzing enzyme present in the serum of adult male rats was in some way connected with the utilization of the body depot fats during starvation. It is evident that significant differences due to previous dietary history may occur within what is usually considered a " normal range " for the enzyme.

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INTRODUCTION

This investigation is concerned with the study of the lipolytic activity of the serum of albino rats, Wistar strain. More than one enzyme may be involved, but for convenience, we will speak of a single enzyme, tributyrinase. Tributyrinase is a member of a group of enzymes known as lipases which in general, hydrolyze the esters of fatty acids and alcohols. The exact function of this enzyme is unknown although its ability to hydrolyze certain fatty acid esters was demonstrated as early as 1896.

The terminology with respect to the lipases is at present confused. This is due mainly to the fact that the lipolytic enzymes are not entirely specific in their action, in the sense that each particular member appears to hydrolyze quite a number of lipid esters. The enzyme which we call serum tributyrinase, for example, splits tripropionin and ethyl butyrate, as well as a variety of other lipid esters. As a result of this variation in specificity different workers propose different names for what appears to be the same enzyme system. The names most commonly used in the literature for the particular enzyme under study are: butyrinase, tributyrinase, non-specific serum esterase, serum esterase, serolipase, and serum lipase. We feel that the only safe practice is to name the enzyme after

the substrate used for assay, therefore, from here on we will use the term "serum tributyrinase".

The problems regarding the origin, physiological function, and exact nature of serum tributyrinase are over half a century old. It was stated by von Hess (39) that Hanriot (15), in 1896, was the first to investigate the origin of "serolipase". The following lines from the paper by von Hess (39) are presented here because they mention early conflicts regarding the nature of "serolipase" and bring to light many features of historical interest.

"THE NATURE OF SEROLIPASE. It is to be noted that most of the work on blood lipases has been done with artificial esters and not true fats. Even Hanriot (15) says, that fats, while acted upon by the blood are not suited for the study of hydrolysis because of their insolubility and small amount of cleavage. In their place he used a fatty acid ester monobutyrin. This is more easily emulsified and split by the blood serum and therefore better adapted for following the course of hydrolysis. Kastle and Loevenhart (19) and Loevenhart (20) also laid stress upon the similarity of action of lipase upon ethylbutyrate and fats.

"Following this a heated discussion arose concerning the lipases of the serum. Arthus (3) and Doyen and Morel (10) claim that the blood serum does not contain a true lipase but only esterases of the lower fatty acids. In 1904 Bitnii-Schljachto (6)

found that serolipase splits artificial fats as easily as monobutyrin. It may be agreed that esterases resemble enzymes in general; but the question now arises whether or not they are identical with true lipases. -".

Strangely enough, 40 years later the problems of emulsification, specificity of the enzyme, choice of substrate, and many others are not settled and are still being argued by the foremost workers in this field.

In 1911, Rona and Michaelis (29) described a method for the estimation of "ester and fat hydrolysis" by the blood and serum. This method was based on the measurement of the amount of surface tension lowering due to the hydrolysis of tributyrin and monobutyrin and the subsequent liberation of butyric acid. Aberhalden and Rona (2) were the first workers who attempted to relate the serum tributyrinase levels to the diet. They reported in 1912 that dogs which had previously been starved and then fed excess fat showed an increase in the serum tributyrinase activity as determined by the method of Rona and Michaelis.

In the same year von Hess (39) showed that in the dog, ligation or extirpation of the pancreas did not alter the concentration of "lipase" in the serum. It is important to note that the substrate used by von Hess was tributyrin, because Cherry and Crandall, in 1932 (8), described a modification of

Loevenhart's method (20) in which they used olive oil as a substrate and demonstrated exactly the opposite results. The latter appear to be the exact antithesis of the findings of von Hess. In recent years, however, many workers have contributed to the currently accepted idea that the lipases of the pancreas and serum are not the same. The fundamental difference is that the serum tributyrinase acts most readily on short chain fatty acid esters, whereas, the lipase of the pancreas shows greatest activity with the esters of long chain fatty acids. The use of these two substrates, tributyrin and olive oil, brings to light the fact that although the pancreatic lipase does increase in the serum after ligation of the pancreatic ducts the serum tributyrinase levels ordinarily present remain essentially unaltered. Furthermore, von Hess demonstrated that not only ligation or extirpation of the pancreas but also ligation of the renal arteries or thyroidectomy had no effect on the serum tributyrinase levels. He concluded that this enzyme had little relationship to the pancreas, thyroid gland, or kidney. This investigator also found that this enzyme is not activated by bile salts. Once again this points to a distinction between serum tributyrinase and pancreatic lipase since the latter enzyme is the classical example of an enzyme activated by bile salts (33), (13).

Further significant contributions to the study of serum

tributyrinase were made in 1912 when Rona and co-workers studied the effects of time and temperature on the activity of the enzyme. It is also of interest to note that in this same year Rona and Ebsen (27) made the first attempt to keep the pH of the reaction medium constant with the aid of phosphate buffers.

Aberhalden stated in 1913 (1) that the lipolytic action of the blood and serum of well nourished dogs increased when the animals were starved. It will be of interest to note later in this thesis the effect of starvation on the serum tributyrinase levels of rats fasted after a number of dietary regimens.

Rona and Bien, in 1914 (26), determined the pH optimum of rabbit serum tributyrinase to be about 8. They stated that serum tributyrinase was different from stomach esterase and probably different from intestinal tributyrinase. They also showed that serum tributyrinase reacts upon different esters with different velocities and suggested that the constitution of the esters was probably the important factor. Later in the same year these workers concluded that the lipases of the pancreas and serum were not identical. Their opinion was based on pH optima studies and differences in the effects produced by calcium, magnesium, barium, manganese salts, and sodium fluoride, on the enzyme activity.

Pietri, in 1915 (23), made the important observation that sodium butyrate retarded the velocity of reaction of the

enzyme under study whereas glycerol had no effect. This indicates that an accumulation of the products of reaction would hinder enzyme activity. The inhibition attributed to sodium butyrate was not due to change in pH. A similar observation has recently been reported by Schoenheyder (32) who worked with pig liver esterase. Pietri also noted that blood letting diminished the enzyme activity. This has recently been confirmed by Tuba and Hoare (38).

Green and Summers reported in 1916 (14), that feeding increased the amount of fat in the blood and to some extent the "lipase" also. They stated, furthermore, that feeding adult dogs after a prolonged fast may result in only a slight rise in blood fat but it would cause a large increase in blood "lipase". They also showed that the effects in puppies differed slightly from those obtained when adult dogs were used.

Forbes and co-workers (12), demonstrated that rats which had previously been starved and then fed various foodstuffs, showed a rise in serum tributyrinase after the ingestion of Oleic and stearic acid and after a meal containing fat. They reported that glycerol, sugar, protein, and Checkers, had little or no effect. Unfortunately their method necessitated the killing of the animals and therefore they were unable to follow the lipase levels for any period of time. It is interesting to note at this point that Bach (4) reported that the fat content of the diet did

not influence the serum tributyrinase levels.

Recently Glotzer and Seligman (13), and others (30,31), have shown, under various normal as well as pathological conditions, the presence in serum of a lipase similar to pancreatic lipase, with respect to velocity of action, activation, and inhibition. Lagerlof (21), has similarly demonstrated what appears to be pancreatic lipase in normal serum. He has also described a method of increasing the pancreatic lipase fraction of the serum with opiates. Lagerlof has pointed out that heparinized plasma and serum show the same activity under a series of varying conditions. He concludes that they contain the same sort of esterase and that it is not physically or chemically bound to the fibrin.

Flock and Bollman (11), reported that the lipolytic esterase content of lymph increases after a meal containing fat, whereas it is unaffected by a fat-free meal. Hess and Viollier (16), on the other hand, state that the tributyrinase content of rat blood plasma is greatly reduced on a fat-free diet.

In view of these findings and preliminary work by Hoare (18), we felt that it was important to study the effects of various dietary regimens on the tributyrinase content of rat serum. In 1949 Tuba and Hoare (36) developed a titrimetric micromethod for the determination of the lipolytic esterase content of small amounts of serum. Previous methods required

such large quantities of blood or serum that small laboratory animals, such as rats, had to be sacrificed for the purpose of determining the serum esterase levels. The method devised by Tuba and Hoare permits the drawing of small amounts of blood from laboratory animals and therefore the enzyme levels can be determined periodically in the same animals. Aside from the obvious advantage of using fewer animals a repetition of runs on the same animal permits one to acquire a larger number of well controlled observations which can then be subjected to statistical analyses similar to those made during our investigations.

EXPERIMENTAL.

A. REAGENTS.

(i) Sodium hydroxide. 0.025N.

One gram of reagent sodium hydroxide was dissolved in 20 ml. of distilled water and diluted to make one litre. This solution was standardized against 0.02N. potassium acid phthalate using phenolphthalein as an indicator.

(ii) Sodium diethylbarbiturate, (Veronal) 0.1M.

10.63 grams of sodium diethyl barbiturate was dissolved in distilled water and diluted to 500 ml.

(iii) Hydrochloric acid 0.1N.

8.69 ml. concentrated reagent hydrochloric acid was diluted to one litre. This solution was standardized against 0.025N. sodium hydroxide using phenolphthalein as an indicator.

(iv) Sodium diethylbarbiturate buffer.

Sodium diethylbarbiturate 0.1M. and hydrochloric acid 0.1N. were added together in quantities sufficient to produce a total of 100 ml. of buffer of pH 8.20. This buffer solution was prepared weekly.

(v) Ethyl alcohol inactivator.

Commercial ethyl alcohol of 95% strength was used.

(vi) Phenolphthalein 0.08%.

80 mgm. of phenolphthalein was dissolved in 1 gram of 95% ethyl alcohol.

(vii) Substrate.

Tributyrin was used directly as obtained from Eastman Kodak Company. Free acid content was not considered significant since this was compensated for in the control determination.

(viii) Alloxan monohydrate.

Alloxan monohydrate obtained from Eastman Kodak Company was dissolved in distilled water as required and injected subcutaneously in doses of 160 mg./kg. of body weight into fasting adult male rats in order to produce alloxan diabetes.

B. DIETARY CONSTITUENTS.

(i) McCollum's salt mixture.

Calcium lactate	35.19%
Calcium biphosphate	14.60%
Potassium phosphate (mono)	25.78%
Sodium phosphate (mono)	9.38%
Sodium chloride	4.67%
Magnesium sulphate (anhydrous)	7.19%
Ferric citrate	3.19%

Use 40 grams / kg. of diet. (4%).

(ii) Vitamin mixture I.

Thiamine hydrochloride	0.5 gm.
Calcium pantothenate	2.0 gm.
Pyridoxine hydrochloride	0.5 gm.
Niacin	0.5 gm.

Made up to 500 ml. with 70% ethyl alcohol.

Use 5 ml. / kg. of diet.

(iii) Vitamin Mixture II.

Powder 298 grams of sucrose and add 2 grams of Riboflavin.

Use 1.5 grams of this mixture / kg. of diet.

(iv) Vitamin Mixture III.

50 grams of choline are made up to 200 ml. with 70% ethyl alcohol.

Use 4 ml. / kg. of diet.

(v) Vitamin Mixture IV.

Vitamin A acetate	20 mgm.
Calciferol	2.3 mgm.
Alpha tocopherol	2 grams.

Dissolve in 5 ml. of diethyl ether and make up to 100 ml. with 95% ethyl alcohol. Use 6 ml. / kg. of diet.

(vi) Casein (commercial).

All diets of Group B. (below) contained 20% commercial grade casein.

(vii) Vitamin free casein ("smaco").

All diets of Group C. (below) contained 20% vitamin free casein.

(viii) Cod liver oil.

Best medicinal cod liver oil. Purchased from W. E. Munn, St. Johns, Newfoundland.

(ix) Crisco.

A hydrogenated vegetable oil product manufactured by Procter and Gamble of Canada, Ltd.

(x) Corn oil.

Distributed by Fisher Scientific Company Limited.

(xi) Raw linseed oil.

Lymans Limited, Montreal, Canada, were the producers.

(xii) Cottonseed oil.

Distributed by Fisher Scientific Company Limited.

(xiii) Olive oil.

Pure imported olive oil, packed by Gattuso Olive Oil Corp., Montreal, Canada.

(xiv) Butter.

Pasteurized creamery butter. "Daisy Brand" first grade, packed by Edmonton City Dairy.

(xv) Oleic acid.

U.S.P. grade. Fisher Scientific Co. Limited.

(xvi) Stearic Acid.

U.S.P. grade. Fisher Scientific Co. Limited.

(xvii) Glycerol.

U.S.P. grade. Fisher Scientific Co. Limited.

(xviii) Sucrose.

Commercial grade.

C. DIETS.

Group A.

Diet 1. Stock laboratory ration, Purina Fox Checkers.

Group B.

All diets in this group were made up in the following manner: 20% commercial grade casein.

4% McCollum's salt mixture.

2% Cod liver oil.

5 ml. / kg. of diet of Vitamin mixture I.

1.5 grams / kg. of diet of Vitamin mixture II.

4 ml. / kg. of diet of Vitamin mixture III.

To these ingredients fat was added in kind and quantity as listed below and the balance of the diet was made up with sucrose.

Diet 2. No fat was added to this diet.

Diet 3. 3% Crisco was added to this diet.

Diet 4. 8% Crisco was added to this diet.

Diet 5. 23% Crisco was added to this diet.

Diet 6. 38% Crisco was added to this diet.

Diet 7. 58% Crisco was added to this diet.

Diet 8. 8% Butter was added to this diet.

Diet 9. 8% Cottonseed oil was added to this diet.

Diet 10. 8% Linseed oil was added to this diet.

Diet 11. 8% Corn oil was added to this diet.

Diet 12. 8% Olive oil was added to this diet.

Diet 13. 23% Butter was added to this diet.

Diet 14. 23% Cottonseed oil was added to this diet.

Diet 15. 23% Linseed oil was added to this diet.

Diet 16. 23% Corn oil was added to this diet.

Diet 17. 23% Olive oil was added to this diet.

Group C.

All the diets in this group were made up in the following manner: 20% vitamin free casein.

4% McCollum's salt mixture.

5 ml. / kg. of diet of Vitamin mixture I.

1.5 grams / kg. of diet of Vitamin mixture II.

4 ml. / kg. of diet of Vitamin mixture III.

6 ml. / kg. of diet of Vitamin mixture IV.

Certain derived lipids or Crisco were added in kind and quantity as listed below and the balance of the diet was made up with sucrose.

Diet 18. Nothing was added to this diet.

Diet 19. The Vitamin mixture IV was omitted from this diet.

Diet 20. 1.040% glycerol was added to this diet.

Diet 21. 9.570% oleic acid was added to this diet.

Diet 22. 9.573% stearic acid was added to this diet.

Diet 23. 10% Crisco was added to this diet.

D. COLLECTION OF RAT SERUM.

Samples of blood were collected between the hours of 8.00 a.m. and 10.00 a.m., as a routine practice. Blood was obtained from the tail by "milking" into specially designed centrifuge tubes; the total amount of blood removed from an individual animal did not exceed 0.4 ml. . The tubes were allowed to stand a few minutes, then they were centrifuged for five minutes at 3000 r.p.m. . The serum was removed by means of a micro-pipette and stored in 5 ml. test tubes. These tubes were stoppered immediately and placed in storage at 5°C. . Determinations were carried out within three days of the time of collection of the sample. Hoare (18) reported that a sample of serum kept over 100 days, at a temperature of 5°C. showed no change in lipolytic activity.

On occasion, particularly at the termination of inanition experiments, animals were decapitated and the blood collected. When rats are fasting or receiving insufficient food they drink very little water and subsequently the blood becomes very viscous. Under these conditions it is very difficult to obtain adequate blood from the tail.

E. MICRO-METHOD FOR THE ESTIMATION OF SERUM TRIBUTYRINASE.

Micro-Kjeldahl tubes of approximately 5 ml. capacity are used for the enzyme determinations. Into one of these tubes are pipetted 0.10ml. serum, 0.20 ml. distilled water, and 1.00 ml. veronal buffer of pH 8.20. The mixture is warmed to 37°C. and then 0.02 ml. of tributyrin, also at 37°C., is added with constant shaking. The contents of the tube are mechanically agitated for 30 minutes at 37°C. on a Warburg shaker at the rate of 120 swings a minute, which maintains the substrate in a finely dispersed state. The pH of the experimental tube at the end of 30 minutes is 7.9 but the average of the initial and terminal pH values is 8.05, which is considered a better representation of the situation.

Serum tributyrinase activity is terminated and the serum proteins are precipitated by the addition of 4.0 ml. of 95% ethyl alcohol. The mixture is transferred to a 10 cm. centrifuge tube and centrifuged for five minutes. The supernatant is poured into a 25 ml. erlenmeyer flask, which is stoppered at once to minimize the absorption of carbon dioxide. The contents of the flask are titrated with 0.025N. sodium hydroxide, using phenolphthalein as an indicator, to a faint but persistent pink color.

A control tube is used which is identical with the experimental tube except that the serum is boiled for 30 seconds before the buffer and substrate are added.

Enzyme activity is measured by the difference between the experimental and the control tubes. The serum tributyrinase activity in units is equivalent to the number of ml. of 0.025N. sodium hydroxide required to neutralize the amount of butyric acid liberated by the enzyme contained in 100 ml. of serum at 37°C. and at pH 8.05. One ml. of 0.025N. base is equivalent to one tributyrinase (Lipase*) unit.

Replicates on the same serum, using this simple and rapid micro-method, have repeatedly shown good agreement well within the limits of 5% experimental error.

* The term "Lipase unit" was previously used by Tuba and Hoare for what we now term a Tributyrinase unit. Three of the graphs presented in this thesis bear this older designation.

F. THE CARE AND FEEDING OF ANIMALS.

In all cases that rats were housed in individual cages and their ears were marked to facilitate identification. Fresh water was available to the animals at all times. They were generally fed ad libitum except during inanition experiments when the animals were either totally starved or placed on graded amounts of food. These exceptions will be dealt with under the topic "Inanition Experiments".

Food consumption was recorded daily for each animal. The food containers were weighed each evening at which time the food was replenished. Since we often fed diets of high fat content the food trays were filled only slightly in excess of the amount of food an animal would eat and the containers were frequently emptied and washed. In this manner we were successful in preventing rancidity and subsequent spoiling of the diets. Food consumption data, as well as being essential to the interpretation of our results, were used as an indication of palatability and by this criterion none of the diets had become spoiled or rancid. Some of the more stable diets were made up every two or three weeks but diets which contained butter were made up once a week. Spillage was taken into account when the food consumption was recorded. All diets were stored in a refrigerator at 5°C. .

All the animals were weighed individually at the beginning of each experiment and at one week intervals thereafter. Growth was generally good and was used as an indication of the general health of the experimental animals.

RESULTS AND DISCUSSION

(I) Relationship between the amount of a stock laboratory ration ingested and serum tributyrinase levels.

In order to determine the effect of total daily food consumption the animals were maintained on a stock laboratory diet of Purina Fox Checkers, which were powdered to minimize scattering by the rats. This diet was fed to normal weanling male rats, normal adult male rats, and alloxan diabetic adult male rats.

(i) In normal weanling male rats and normal adult male rats.

Six weanling male rats, 21 days old, and six adult male rats were placed on the diet of powdered Checkers, which they received ad libitum for six weeks. The means of the results for these two experiments are given in Table I. There is no correlation between daily food consumption and serum tributyrinase activity in either growing or mature animals ($\underline{r} = 0.06$ and 0.01 respectively). There is, however, a highly significant correlation between the weights of the animals and tributyrinase levels in the weanling group ($r = 0.56$). This further confirms the observations of Tuba and Hoare (37) that serum tributyrinase levels increase from low levels at birth to higher levels which become constant in the mature animal. The lack of correlation

between the weights of the adult group and their serum tributyrinase levels ($r = 0.16$) is therefore to be expected.

(ii) In alloxan diabetic male rats.

Twenty adult male rats were fasted overnight and each was injected subcutaneously with 160 mgm. of alloxan monohydrate per kg. of body weight. Fourteen animals died within twenty four hours. The remaining six rats were very definitely diabetic as shown by a pronounced hyperglycemia with blood sugar values ranging from 300 to 500 mgm. per hundred ml. of blood. The blood sugars were determined by the method of Reinecke (24). Pronounced glycosuria, polyuria, and polydypsia were also evident and the blood was thick and dark suggesting hemoconcentration.

These six rats, were maintained on a diet of powdered Checkers for a period of six weeks to allow the diabetic state to become fully established and the serum tributyrinase levels to become stabilized. Hoare reported (18) that the serum tributyrinase levels increased to about the sixteenth day when a steady high range was reached. At the end of the pre-experimental feeding period the mean serum tributyrinase activity for the group was well above normal, in accordance with the findings of Tuba and Hoare (37). While on experiment, the rats were fed powdered Checkers for six days, the amount of food eaten by each animal was measured daily, and the serum tributyrinase (lipase) activity was

estimated every two days. The mean tributyrinase values are given in Table II as well as the mean average food consumption for the 24 hours immediately preceding the bleeding for determination of tributyrinase levels.

The regression line shown in Figure 1 was calculated from the 3 tributyrinase values and the 6 daily consumption levels determined for each rat during the course of the above experiment. The calculated equation for this regression line is $T = 44C - 640$ (T = tributyrinase in units/100 ml. serum and C = food consumption in gm./day/rat) and this has a highly significant t value = 3.1 ($P < 0.01$). This together with the value of $r = 0.64$ for the data of Table II, indicates that there is a highly significant correlation between food ingested and serum tributyrinase concentration in alloxan diabetic adult male rats.

A simple arithmetical comparison of the consumption and enzyme data of the preceding two tables seems to indicate that the abnormally high tributyrinase activity associated with alloxan diabetes in rats is largely attributable to the increase in food consumption. This can readily be proved statistically. We have used the individual consumption and tributyrinase data for six alloxan diabetic rats (Table II) and for six normal adult rats (last 3 weeks in Table I) and there is a highly significant correlation. $r = 0.63$ with a t value of 3.59 at the 1% level.

The lack of correlation in normal adult rats eating unrestricted amounts of powdered Checkers is probably due to the wide fluctuations in the activity of the enzyme within a group of animals eating much the same quantity of food from day to day and from week to week (Table I). Only when both consumption and tributyrinase vary markedly from the normal ranges, as in the alloxan diabetic group (Table II) is this correlation readily shown.

The fact that a correlation between food intake and serum tributyrinase concentration could be obtained under certain conditions led to a consideration of the effect on the enzyme of various dietary constituents, notably fat. In this regard, Hess and Viollier (16) have reported that the absence of fat from the diet reduces the serum tributyrinase content of rat plasma.

TABLE I

THE RELATIONSHIP BETWEEN DAILY FOOD CONSUMPTION AND SERUM LIPASE (TRIBUTYRINASE) LEVELS IN WEANLING AND ADULT MALE RATS RECEIVING A DIET OF POWDERED ANIMAL CHECKERS

(Averages for six animals in each group)

Time after beginning of experiment	Weanling Rats			Adult Rats	
	Consumption in gm./day/rat	Serum lipase, units/100 ml.	Weights in grams	Consumption in gm./day/rat	Serum lipase, units/100 ml.
1 week	12.0± 0.5*	--------	73± 5*	22.0± 0.5*	760± 30*
2 weeks	15.0± 0.5	510± 30*	113± 6	22.0± 0.5	610± 40
3 "	18.0± 0.5	610± 10	156± 7	21.0± 0.5	560± 20
4 "	19.0± 0.5	590± 10	187± 5	22.0± 0.5	580± 10
5 "	20.5± 0.5	600± 20	228± 6	21.0± 0.5	680± 30
6 "	20.0± 0.5	610± 20	240± 5	20.0± 0.5	660± 40

* Standard error of the mean

TABLE II

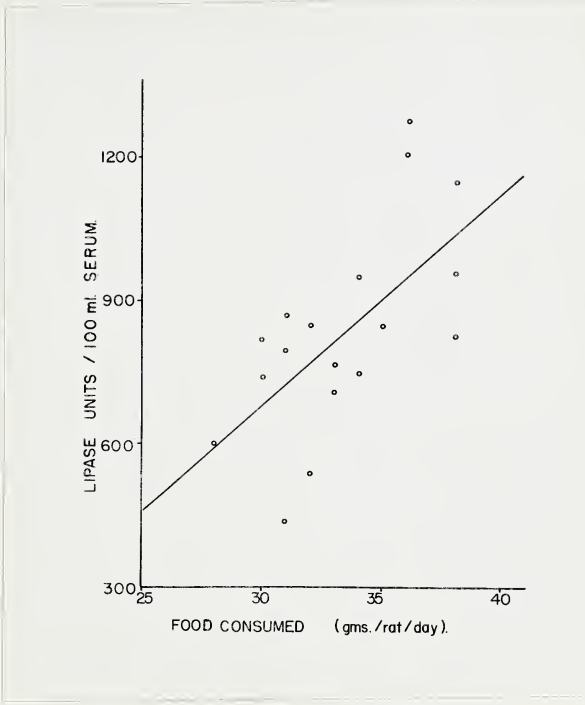
THE RELATIONSHIP BETWEEN DAILY FOOD CONSUMPTION AND SERUM LIPASE (TRIBUTYRINASE) LEVELS IN ALLOXAN DIABETIC ADULT MALE RATS

(Averages are for six animals in the group)

Time after beginning of experiment	Consumption in gms./day/rat	Serum lipase in units/100 ml.
2 days	32± 1.3*	800± 60*
4 "	35± 1.2	890± 100
6 "	33± 0.9	810± 100

* Standard error of the mean

Figure 1.



Relationship between the serum tributyrinase (lipase) levels and consumption of Purina Fox Checkers by alloxan diabetic adult male rats.

The equation of the regression line is:

$$T = 44C - 640.$$

(II.) The effect of dietary hydrogenated vegetable fat on the serum tributyrinase levels and growth of male rats.

(i) On the serum tributyrinase levels of weanling male rats.

Groups of ten weanling male rats were fed ad libitum for six week periods on a number of synthetic diets containing the various levels of fat indicated in Table III. Each diet (diets 3 to 7 inclusive) contained 2% cod liver oil and the balance of the fat was made up with Crisco. Tributyrinase levels were estimated weekly except at the end of the first week, when the animals were too small to bleed. The daily food intake of individual animals was variable on some of the diets, especially when higher concentrations of fat were fed. Consequently the values for food consumption reported in Table III in grams eaten per rat per day are the means of the total food eaten by the ten animals of each group over a period of one week. From the food consumption data the amount of fat eaten per rat per day has been calculated.

A significant correlation exists between the amount of fat eaten daily by weanling rats and the levels of serum tributyrinase. The value of $r = 0.71$. The formula of the regression line shown in Figure 2 is $T = -33F + 540$ (T = tributyrinase in units/100 ml. serum and F = fat consumption in gm./day/rat.) and this has a highly significant value of $t = 5.2$. The standard error of the estimate for the line is ± 51.

(ii) The effect on the serum tributyrinase levels of adult male rats.

The preceding experiment was repeated with a group of ten normal adult male rats. Tributyrinase activities were determined at the end of each week of the feeding period. As with the weanling animals the average daily consumption of food became less as the fat concentration of the diets was increased. The daily consumption of fat once more was higher as the dietary fat concentration was increased. A highly significant correlation exists between the consumption and enzyme data given in Table IV. ($\underline{r} = 0.78$).

The formula of the regression line shown in Figure 3 is $T = 33F + 390$ (T = tributyrinase in units/100 ml. serum and F = fat consumption in gm./day/rat.) and this has a highly significant $\underline{t}$ value of 6.9. The standard error of the estimate for this regression line is $\pm$ 55.

A striking fact concerning the regression lines for the above two experiments is that the slopes have opposite signs. The increased lipolytic activity of serum associated with increased fat ingestion in the case of adult animals could be expected if serum tributyrinase has a role in the metabolism of fats. The findings with weanling animals is surprising. The data in Table V offers some explanation.

(iii) Growth of weanling male rats on hydrogenated vegetable fat.

The growth of weanling rats fed the various diets listed in Table V was best on Checkers, which contained about 5% fat. Growth on the synthetic diets containing 5 and 10% fat was not as good, but they were not as palatable as the Checkers, judging by consumption data. This diminished rate of growth can be accounted for on the basis of lowered daily food consumption which in 9 week old rats (at the end of the 6 week feeding periods) ranges from 20 grams on the Checker diet to 6.2 grams on the 60% fat diet. The synthetic diets which contained more than 5% fat not only interfered with the rate of growth, because of inadequate protein intake, but tended to maintain the mean tributyrinase levels near the values of 320 to 480 units per 100 ml. reported by Tuba and Hoare (37) for 3 to 4 week old rats.

After the termination of the feeding experiments, each group of animals was placed on Checkers for one week. An improvement in the rate of growth was noted in all but one group, and the enzyme levels increased toward the range of values characteristic of the stock laboratory diet.

Deuel and his co-workers (9) found that rats fed ad libitum on synthetic diets containing 5 to 50% fat showed better growth than those fed lower concentrations of fat or a stock laboratory diet. Their results were similar for cottonseed oil and a hydrogenated vegetable fat, margarine.

Barki, Collins, Elvehjem, and Hart (5) fed various levels of three vegetable oils and butter to growing rats and observed that the dietary concentration of each for optimal growth was characteristic of the fat. These levels were 10% for corn oil and 35% for butter fat, whereas coconut oil and soybean oil concentrations had no effect on growth. In our experiments, which were primarily designed for tributyrinase studies, the closest approximation to both "normal" growth and serum tributyrinase activity was obtained with the synthetic diet containing 5% fat. Our results are probably affected to some extent by the fact that each synthetic diet contained 2% cod liver oil.

TABLE III

THE EFFECT OF DIETARY FAT CONCENTRATION ON MEAN DAILY FOOD CONSUMPTION (GM. PER DAY)(C), MEAN DAILY FAT CONSUMPTION (GM. PER DAY)(F), AND SERUM TRIBUTYRINASE (UNITS PER 100 ML.)(T) OF WEANLING MALE RATS

(Averages are for ten animals in each group)

Fat in diet, %	2 weeks			3 weeks			4 weeks			5 weeks			6 weeks		
	C	F	T	C	F	T	C	F	T	C	F	T	C	F	T
5	11.0	0.55	530	10.8	0.54	550	11.4	0.57	540	14.1	0.70	570	14.1	0.70	530
10	8.2	0.82	550	9.1	0.91	500	12.0	1.20	470	12.3	1.23	470	10.0	1.00	470
25	10.2	2.53	510	10.2	2.53	480	13.5	3.38	530	10.2	2.53	540	12.0	3.00	410
40	6.0	2.40	380	7.8	3.12	440	7.5	3.00	370	12.4	4.97	450	14.0	5.52	330
60	6.1	3.96	400	7.0	4.20	430	8.3	4.98	370	7.6	4.56	400	6.2	3.72	300

TABLE IV

THE EFFECT OF DIETARY FAT CONCENTRATION ON MEAN DAILY FOOD CONSUMPTION (GM. PER DAY)(C), MEAN DAILY FAT CONSUMPTION (GM. PER DAY)(F), AND SERUM TRIBUTYRINASE (UNITS PER 100 ML.)(T) OF ADULT MALE RATS

(Averages are for ten animals in each group)

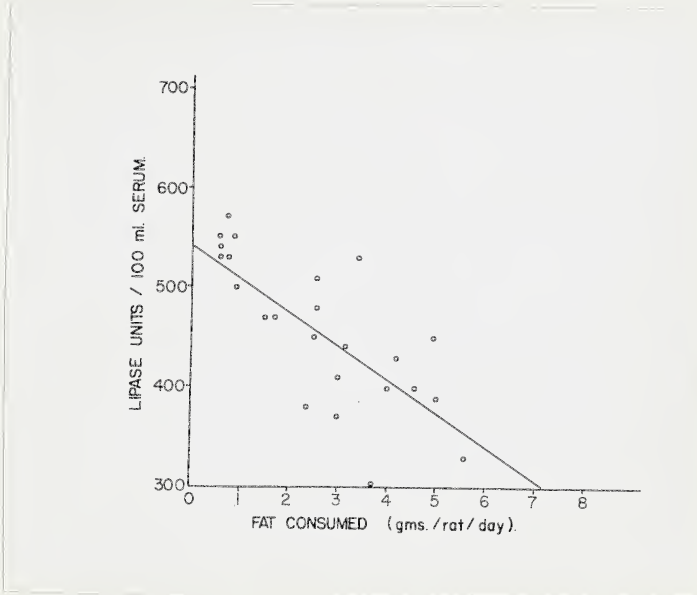
Fat in diet, %	1 week			2 weeks			3 weeks			4 weeks			5 weeks			6 weeks		
	C	F	T	C	F	T	C	F	T	C	F	T	C	F	T	C	F	T
5	14.1	0.71	400	13.6	0.68	410	11.4	0.57	440	12.0	0.60	470	14.6	0.73	420	15.0	0.75	450
10	14.7	1.47	410	13.0	1.30	370	12.0	1.20	470	15.0	1.50	420	14.8	1.48	400	14.6	1.46	380
25	15.2	3.80	520	12.6	3.15	520	11.8	2.95	540	11.4	2.85	430	11.3	2.82	440	11.2	2.80	440
40	11.4	4.56	570	11.8	4.72	650	8.8	3.52	660	11.8	4.72	590	12.3	4.92	640	12.0	4.80	660
60	13.6	8.16	590	9.1	5.45	590	8.4	5.04	550	10.4	6.25	500	9.5	5.70	540	9.7	5.83	550

TABLE V

THE RELATIONSHIP BETWEEN AVERAGE GROWTH RATE OF WEANLING MALE RATS (IN GM.) ON VARIOUS DIETARY CONCENTRATIONS OF FAT AND AVERAGE SERUM TRIBUTYRINASE ACTIVITY (T) (UNITS PER 100 ML.)

Diet		Weight after 6 weeks on diet	Weight gain in 6 weeks	"T" at end of 6 weeks	Weight gain after one week on Checkers	"T" after one week on Checkers	Change in "T" produced by Checkers
1	Checkers (5% fat)	240	185	610	30	---	---
3	Crisco (5% fat)	213	142	570	40	610	+40
4	" (10% ")	201	149	470	7	550	+80
5	" (15% ")	180	121	410	31	660	+250
6	" (40% ")	183	138	330	30	560	+230
7	" (60% ")	147	92	300	41	420	+120

Figure 2.

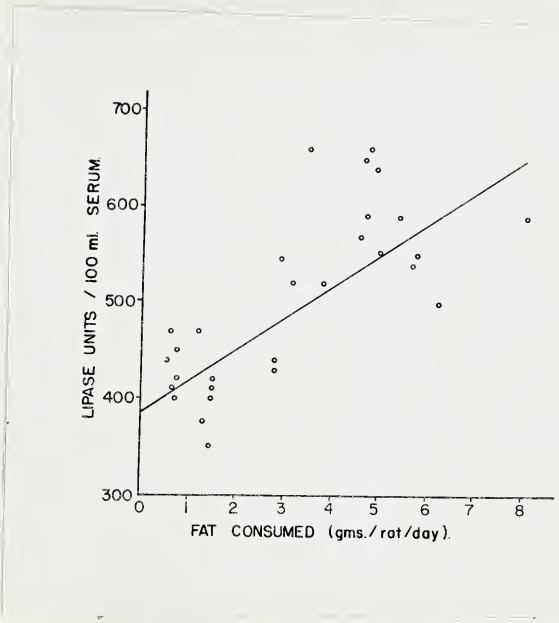


Relationship between the serum tributyrinase (lipase) levels and consumption of Crisco by weanling male rats.

The equation for the regression line is:

$$T = -33F + 540.$$

Figure 3.



Relationship between the serum tributyrinase (lipase) levels and consumption of Crisco by adult male rats.

The equation for the regression line is:

$$T = 33F + 390.$$

(III) The effects of some fats and oils on the serum tributyrinase levels of adult male rats.

After we had shown that the amount of Crisco ingested could be related to the serum tributyrinase levels we decided to extend the investigation to include other dietary fats. Furthermore, we wished to find out if differences in composition of these fats would give rise to variations in the lipolytic activity of rat serum. Accordingly we selected the following fats and oils: butter, cottonseed oil, linseed oil, corn oil, and olive oil. Butter was chosen because it has the most constant composition of any animal fat and because it contains a large proportion of short chain fatty acids. The vegetable oils varied in the amounts and kinds of fatty acids which they contained and in their tocopherol content.

Ten groups, each containing six adult male rats, were fed Checkers ad libitum for one week. At the end of this pre-experimental feeding period the initial tributyrinase values were determined (Table VI) and the animals were weighed. They were then fed diets 8 to 17 for a period of four weeks. The mean tributyrinase levels, mean changes in body weight, and the mean food consumption, for each group during the four week test period are listed in Table VI.

Studies on diets 4 and 5, which were conducted as a part of the previous experiment on the effect of a hydrogenated vegetable fat on the serum tributyrinase levels of adult male

rats, are also included for comparison in this section. To simplify the statistical analyses and graphs the serum tributyrinase values for four weeks only and for the first six rats of each of the two groups on the Crisco diets are considered.

Statistical analysis of the food consumption data shows that no significant differences exist between the six groups of animals fed diets containing 8% fat and the same is true for the six groups of rats fed diets containing 23% fat. There is of course a significant difference in the amount of fat ingested by the six groups of rats eating the 8% fat diets and the six groups receiving the 23% fat diets.

An analysis of variance of the serum tributyrinase levels for all twelve groups of rats indicates that many significant differences exist. The calculated minimum significant difference is ± 40 tributyrinase units per 100 ml. serum. This means that if the difference between the mean values for any two groups of rats during the four week test period exceeds 40 tributyrinase units then the two groups can be said to differ significantly from each other.

It can be seen from Table VI., that for each fat fed, a significant difference in tributyrinase levels exists between each group of rats eating a diet containing 8% fat and the group eating 23% of the same fat. This can be accounted for on the basis of differences in the amount of fat ingested. Other

significant differences in the tributyrinase levels can be detected which cannot be accounted for on the basis of variations in fat consumption. For example, if we consider the six groups of rats on the diets containing 8% fat we can see that Crisco does not differ significantly from butter as far as the effect on serum tributyrinase is concerned but it does differ significantly from all the other fats. Furthermore, it can be seen that the effect of butter differs significantly from every other fat which was fed with the exception of Crisco. These variations can be accounted for only on the basis of dissimilarities in the composition of the fats. At the 23% fat levels fewer significant differences exist and this suggests that there may be a dietary concentration of fat below 23% for which the serum tributyrinase shows its maximum response.

It can be seen from Table VI., that on the basis of decreasing magnitude of tributyrinase levels the various fats tested can be arranged in the following order: Crisco, butter, cottonseed oil, linseed oil, corn oil, and olive oil. It might be noted that at the 23% fat level the mean serum tributyrinase value for diet 13, which contained butter, was much lower than it should have been according to the position it has been given in the order listed above. This apparent discrepancy may be accounted for by the fact that butter contains some 15% water

as well as traces of other non-lipid materials. The difference is not great at the 8% fat level but it is magnified almost threefold at the 23% fat level. If this is corrected for, then butter may occupy the position given it in Table VI.

We could find no single factor on the basis of which the above results could be explained. The order of the serum tributyrinase values parallels the total molar fatty acid content more closely than any other single variable to which they were compared. Our endeavours along this line led us to believe that probably there may be a number of factors involved which might affect the relationship between the amount of fat eaten and the serum tributyrinase levels.

TABLE VI

EFFECTS OF VARIOUS DIETS CONTAINING 8% AND 23% FAT ON THE SERUM TRIBUTYRINASE (UNITS/100 ML. SERUM)(T), DAILY FOOD CONSUMPTION (GRAMS)(C), AND ON BODY WEIGHTS (GRAMS)(△W) OF ADULT MALE RATS. (MEANS OF SIX ANIMALS)

Diet	Type of Fat and Dietary Concentration	Zero Values (T)	Means of Four Weekly Values During Test (T)	(△W)	(C)
4	Crisco 8%	660 ± 20*	410 ± 10	+62 ± 11	13.7 ± 0.5
5	Crisco 23%	660 ± 20	520 ± 10	+58 ± 10	12.7 ± 0.5
8	Butter 8%	560 ± 20	380 ± 10	+43 ± 5	14.5 ± 0.4
13	Butter 23%	640 ± 10	420 ± 10	+45 ± 6	13.5 ± 0.7
9	Cottonseed oil 8%	600 ± 40	340 ± 10	+44 ± 5	15.6 ± 0.5
14	Cottonseed oil 23%	760 ± 20	500 ± 10	+59 ± 5	14.2 ± 0.4
10	Linseed oil 8%	580 ± 20	330 ± 10	+24 ± 5	14.4 ± 0.4
15	Linseed oil 23%	710 ± 30	460 ± 20	+48 ± 6	13.7 ± 0.6
11	Corn oil 8%	630 ± 20	320 ± 10	+34 ± 7	14.8 ± 0.5
16	Corn oil 23%	790 ± 50	450 ± 20	+54 ± 9	12.5 ± 0.3
12	Olive oil 8%	610 ± 30	290 ± 10	+28 ± 4	17.1 ± 0.6
17	Olive oil 23%	750 ± 30	450 ± 10	+56 ± 5	13.9 ± 0.5

* Standard error of mean.

Minimum significant difference = ± 40 Tributyrinase (Units/100 ml. Serum).

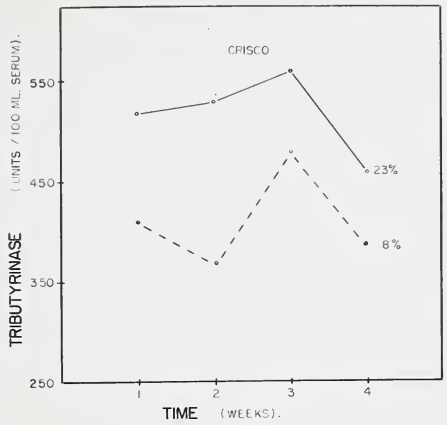
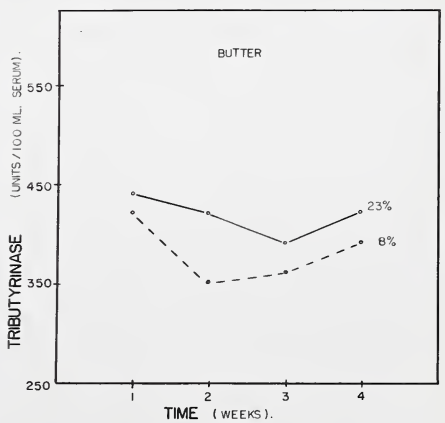


Figure 4. The effect of dietary Crisco (diets 4 and 5) on the serum tributyrinase levels of adult male rats.

Figure 5. The effect of dietary butter (diets 8 and 13) on the serum tributyrinase levels of adult male rats.



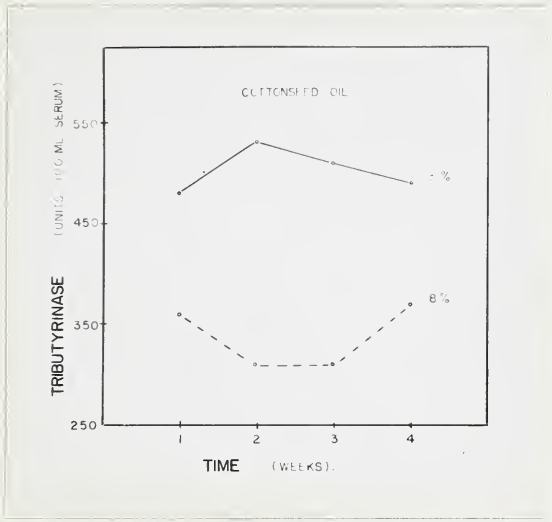
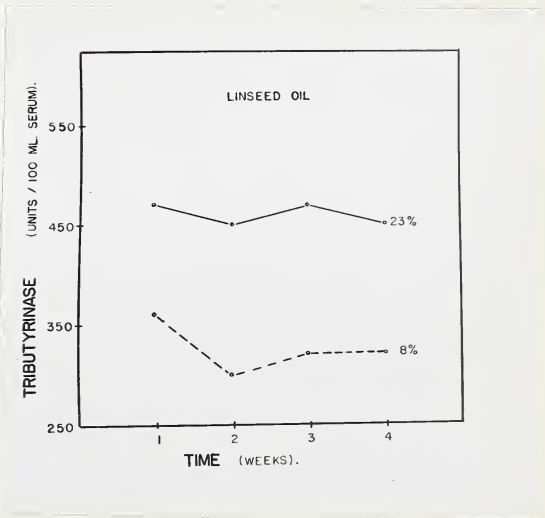


Figure 6. The effect of dietary cottonseed oil (diets 9 and 14) on the serum tributyrinase levels of adult male rats.

Figure 7. The effect of dietary linseed oil (diets 10 and 15) on the serum tributyrinase levels of adult male rats.



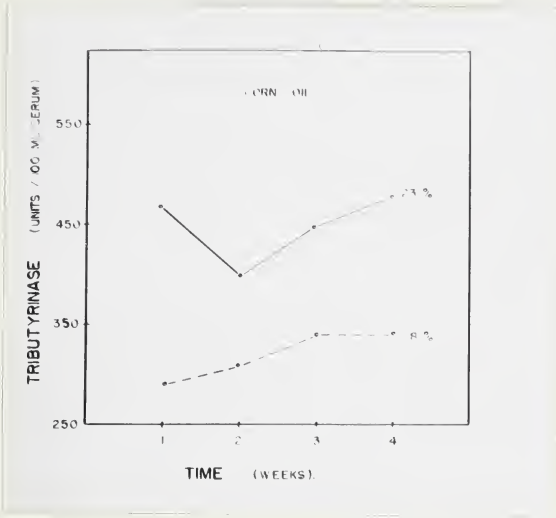
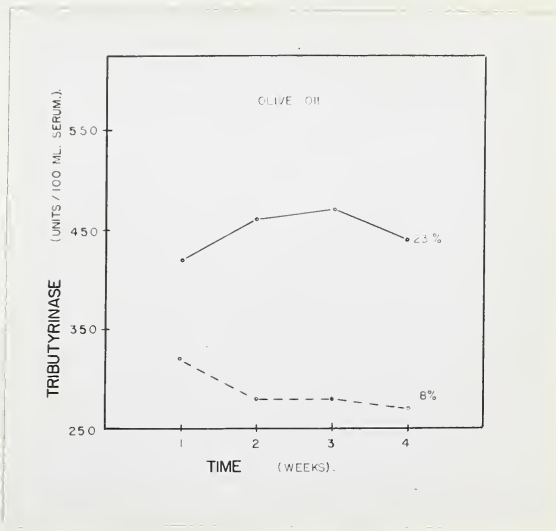


Figure 8. The effect of dietary corn oil (diets 11 and 16) on the serum tributyrinase levels of adult male rats.

Figure 9. The effect of dietary olive oil (diets 12 and 17) on the serum tributyrinase levels of adult male rats.



(IV) The effects of some derived lipids and fat soluble vitamins on the serum tributyrinase levels of adult male rats.

Our previous experiments have shown that both the amount and kind of fat ingested influence the serum tributyrinase levels of adult male rats. A careful scrutiny of the results obtained by feeding various kinds of fats indicated that perhaps the total number of moles of fatty acids ingested might be the principle factor responsible for the alterations in the serum tributyrinase content of rat serum. It has been stated by various workers (33), (34), that some of the fat soluble vitamins affect the levels of various esterases of the serum. Hess and Viollier in 1948 (16), and other authors (7), have shown that in E avitaminotic female rats the cholinesterase activity was below normal. We felt, therefore, that we should also test the effects of ingestion of the fat soluble vitamins A, D, and E, on the serum tributyrinase levels.

Adult male rats weighing at least 250 grams were housed in individual cages and given food and water ad libitum. The daily food consumption, body weight, and levels of serum tributyrinase were determined for each animal after they had received Purina Fox Checkers for one week. After these initial values had been established, the animals were placed in groups of six on the diets indicated below, for periods of four weeks,

and at the end of each week the various experimental data were again obtained, as well as at the end of the fifth week during which interval of time the animals were starved.

The rats were fed diets 18 to 23 inclusive, during the four week test period. Diet 18 was a control diet which was fat free but it contained the fat soluble vitamins A, D, and E. Diet 23 was also a control diet and in addition to the fat soluble vitamins mentioned above it contained 10% Crisco. Diet 19 consisted of the basal control diet, with the crystalline vitamins A, D, and E omitted, in order to estimate the effect, if any, of these three substances on the serum enzyme. The remainder of the diets were devised to test the effect on the serum tributyrinase of the glycerol, oleic acid, or stearic acid, present in a diet containing 10% triglyceride. Diets 20 and 22 contained an amount of glycerol and stearic acid, respectively, that would be equivalent to that present in a 10% tristearin diet. Diet 21 contained an amount of oleic acid which would be equivalent to that found in a 10% triolein diet.

The effects of the six diets on the levels of serum tributyrinase are indicated in Table VII. Enzyme levels at zero time and means of the four weekly values obtained during the test period are presented, as well as the average daily food consumption and changes in body weight. The changes in body

weight which occurred as a result of feeding each diet for four weeks show that in every case the animals gained weight. Weekly fluctuations in the serum tributyrinase levels are shown in figure 10.

The weight gains of the six groups of animals after four weeks on the diets were not significantly different from each other, despite the fact that the first three diets were fat free. This may be taken as an indication that the food consumption was adequate and that the animals were in good health in every case. The animals must also have had ample reserves of the three fat soluble vitamins A, D, and E, and of the essential fatty acids to carry them through the test period when they were receiving diets lacking these nutritional factors. There are no significant differences in food consumption on the basis of which the enzyme levels can be interpreted.

The serum tributyrinase values of the animals receiving the first three diets are the lowest of the six groups, and they do not differ significantly from each other. A fat free diet, therefore, results in a profound decrease in enzyme activity from the levels found in animals receiving the stock laboratory ration. This change is not modified by the absence of the fat soluble vitamins A, D, and E, (diet 19) or by the addition of glycerol (diet 20).

Stearic acid (diet 22) and Crisco (diet 23) produce

serum tributyrinase activity which is in each case significantly greater than what may be considered a basal value established by the three fat free diets. The enzyme levels for diets 22 and 23 do not differ significantly from each other. It is of importance to note that values for these two diets are not as great as those obtained with all six groups during the initial period on Purina Fox Checkers. Since the Checkers contain approximately 5% total "fat", there must be present lipids or other factors which effect the serum tributyrinase activity, and therefore levels of the enzyme in the serum of animals receiving this diet cannot be accounted for on the basis of the ingestion of triglycerides or saturated fatty acids alone.

The use of oleic acid (diet 21) as a supplement to the basal diet produced tributyrinase levels which were significantly greater than those obtained for any of the other groups that were tested. However, in spite of the fact that fatty acid equivalent to 10% triglyceride was present in the diet, enzyme activity was not as high as with Checkers.

It was possible to conclude from this experiment that the ingestion of the fat soluble vitamins A, D, and E, does not affect the serum tributyrinase levels to any appreciable extent. The same appears to be true for glycerol. It is difficult to draw any concrete conclusions regarding the stearic and oleic acids because our previous experiments (diets 8 to 17) seem to

indicate that diets of high oleic acid content should produce lower enzyme levels than the diets rich in unsaturated fatty acids, if they are to have an effect at all. Perhaps further studies involving other fatty acids varying in chain length and degree of saturation might throw some light on this problem.

TABLE VII

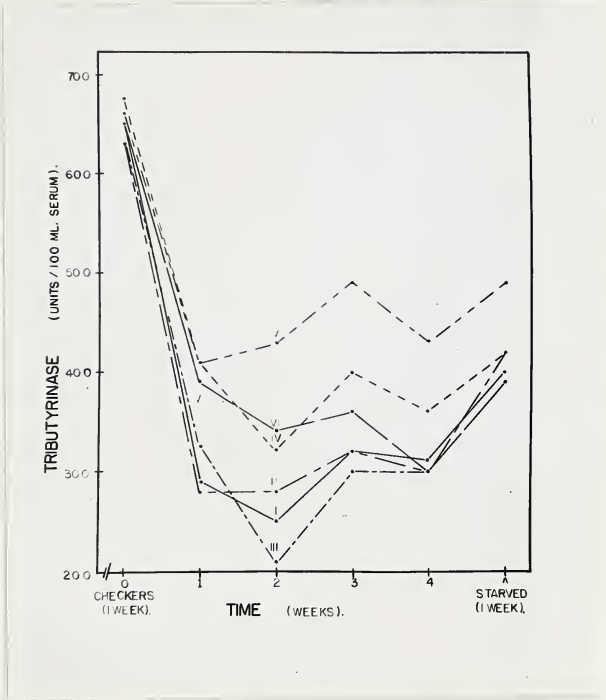
THE EFFECTS OF DIETS 18 - 23 ON SERUM TRIBUTYRINASE (UNITS/100 ML. SERUM)(T), DAILY FOOD CONSUMPTION (GRAMS)(C), AND ON BODY WEIGHTS (GRAMS)(Δ W) OF ADULT MALE RATS. (MEANS OF SIX ANIMALS).

Diet	Zero Values (T)	Means of Four Weekly Values During Test (T)	(Δ W)	(C)
18	650 ± 40*	290 ± 10	52 ± 7	13.8 ± 0.5
19	630 ± 30	290 ± 10	38 ± 7	14.0 ± 0.5
20	630 ± 20	280 ± 10	37 ± 4	15.5 ± 0.4
21	650 ± 20	440 ± 10	50 ± 6	13.6 ± 0.4
22	680 ± 20	370 ± 20	31 ± 4	12.8 ± 0.4
23	660 ± 20	350 ± 10	46 ± 3	13.5 ± 0.3

* Standard Error of the Mean.

Minimum significant difference ± 40 tributyrinase (Units/100 Ml. Serum).

Figure 10.



The effects of some derived lipids and fat soluble vitamins on the serum tributyrinase levels of adult male rats.

(Diets 18 to 23).

Diet 18 ..... I.	Diet 21 ..... V.
Diet 19..... II.	Diet 22 ..... VI.
Diet 20 ..... III.	Diet 23 ..... VI.

(V.) The effect of dietary cod liver oil.

Two groups of male rats were fed diet 2, which contained 2% cod liver oil, for six weeks and their tributyrinase levels were determined weekly. The first group consisted of six adult male rats and the second group contained six weanling male rats. In both cases the enzyme levels were very low and although this is to be expected in the adult animals it is surprising, in the light of our experiments with Crisco, to find such low values in weanling rats. The regression line for this experiment, (figure 2) might lead us to expect higher enzyme levels in the case of weanling rats on diets low in fat, such as 2% cod liver oil.

In the experiment in which the fat soluble vitamins were added to diets free of fat (diets 18 and 20) the tributyrinase levels for adult male rats were lower than those obtained with diet 2 which contained only 2% cod liver oil. Diets 2 and 19 were fed to adult male rats and the resulting tributyrinase levels for diet 2 (Table XI) were significantly higher than the values obtained with diet 19 (Table VII). This is an important point to keep in mind when conducting further dietary studies. It is undoubtedly a fact that the cod liver oil in various diets modifies the effect of other dietary fats on the serum tributyrinase levels. This modifying effect may be eliminated by adding concentrates of the fat soluble vitamins or by feeding the purified vitamins as we did with diets 18 to 23.

(VI) Inanition experiments.

Tuba and Hoare (37) reported that adult male rats which had been starved for a period of one week showed virtually no change in their serum tributyrinase levels. This observation appears unusual in the light of results which were presented earlier in this thesis with regard to the experiments on diets of graded fat content and fat free diets. Our findings suggested that the tributyrinase levels of adult male rats should be very low when the animals were completely deprived of food and hence of dietary fat (Table VII and Figure 10). This inconsistancy between the tributyrinase levels of starved rats and the animals on diets free of fat prompted us to investigate further the effects of starvation from several different viewpoints.

(i) The effects of prolonged starvation on the serum tributyrinase levels of adult male rats.

Because Tuba and Hoare starved their animals for only one week we thought that perhaps the serum tributyrinase response could not be demonstrated in this length of time and therefore we starved the animals until a few had died. Six adult male rats were fed powdered Purina Fox Checkers ad libitum for one week and their normal tributyrinase levels were determined. They were then starved and the enzyme assays were repeated on the

seventh, eleventh, and fourteenth days. Table VIII shows the weights and tributyrinase levels for these animals over this test period. Green and Summers reported (14) that there is an increase in the serum "lipase" levels of dogs during prolonged starvation. Our results showed a slight increase in the serum tributyrinase levels of adult male rats on the seventh day but this elevation is not statistically significant. We found, however, that starvation beyond eleven days lowered the serum tributyrinase levels and that this decrease is significantly different from the Checker values. It is interesting to note that two of the animals succumbed after the bleeding on the fourteenth day. When animals were returned to ad libitum feeding on a Checker diet, for one week, the serum tributyrinase levels were raised to the "normal" range for this ration.

(ii) The effect of limiting food intake on the serum tributyrinase levels of adult male rats.

Complete deprivation of food did not reduce the serum tributyrinase levels until the animals were near death and therefore we thought that it might be possible to lower the enzyme levels by limiting the food intake of a group of animals. This experiment was also intended as a supplement to the experiments in which we attempted to correlate the

food intake with the serum tributyrinase levels of rats on a diet of powdered Purina Fox Checkers. We felt that perhaps the variation in serum tributyrinase levels within a group of adult male rats was so great that the small normal differences in fat consumption on a diet which only contained 5.2% fat, would not be sufficient to produce a detectable change.

Six mature male rats which had been eating powdered Checkers ad libitum for several weeks were then limited to 15 grams each daily for one week. During the following four weeks they were maintained on daily levels of 12, 9, 6, and 3 grams of food per day for one week on each successive decrement. The mean serum tributyrinase levels remained within the normal limits for the first four weeks of the experiment. Only at the end of the week on a daily intake of 3 grams of food did the results given in Table IX show a decline from normal serum tributyrinase concentrations. We were surprised to find that the enzyme levels in this experiment could not be correlated to food consumption. This brings to light a very important point. The serum tributyrinase is not only connected with the ingestion of fat but it would also appear to be associated in some manner with the utilization of body stores of lipids. Once again as might be expected the weights of the animals and the activity of the enzyme showed no correlation.

(iii) The effects of post-dietary starvation on the serum tributyrinase levels of adult male rats after feeding various synthetic diets.

The previous inanition experiments seem to indicate that starvation exerts a special effect on the serum tributyrinase levels of adult male rats. We felt that some new light might be thrown on in vivo lipolysis if rats were starved after feeding various synthetic diets containing known kinds and amounts of fats. The effects of post-dietary starvation on the serum tributyrinase, following the feeding of twelve synthetic diets, are indicated in Table X. Enzyme levels for zero time, and means for the four weekly values obtained during the test periods are presented again (see also Tables VI and VII) for comparative purposes, as well as the initial mean weights and changes in body weight. It should be noted that there are large variations in the amount of body weight gained on the various diets while the amount of weight lost in all the starvation experiments is relatively constant. Since only adult male rats were used the weight gains during the experimental period can be attributed to increased deposition of fat. During the post-experimental starvation period the fat depots would be used by the animals as a source of energy.

The starvation values obtained for each group of animals may be compared with the aid of the calculated minimum

significant difference, which was ± 40 tributyrinase units per 100 ml. serum. Starvation following four weeks feeding on the two diets which contained butter (diets 8 and 13) produced slightly lowered tributyrinase levels which were barely significant. Total deprivation of food after feeding diet 10, which contained linseed oil, failed to elevate the tributyrinase levels significantly. Post-dietary fasting after the test periods on all the other diets resulted in significant elevations of the tributyrinase levels. The increases in serum tributyrinase levels for the two groups of rats which had been starved after feeding on diets 21 and 23, which contained oleic acid and Crisco respectively, are just barely significant. The increase in tributyrinase activity, which resulted from starvation of the group of animals that were fed the stearic acid diet, was just significant. All the other elevations are either very significant of highly significant.

The above experiment indicates that the previous dietary history may affect the tributyrinase levels produced during starvation. This would appear logical since several workers have demonstrated that the composition of the depot fat depends to some extent on the type of fat fed an animal (17). It is curious that an enzyme which splits tributyrin should be so intimately concerned with oleic acid. This problem remains for further investigation.

TABLE VIII

MEAN WEIGHTS AND SERUM TRIBUTYRINASE (UNITS/100 ML. SERUM)(T) LEVELS OF ADULT MALE RATS DURING PROLONGED STARVATION

	Mean Tributyrinase	Mean Weight
Checkers.**	580 ± 30*	271 ± 9*
Starvation 7 days.**	620 ± 20	213 ± 7
Starvation 11 days.**	560 ± 30	183 ± 7
Starvation 14 days.**	330 ± 20	164 ± 8
Post-dietary Checkers 7 days.***	510 ± 30	264 ± 7

* Standard Error of the Mean.

** Mean values of six animals.

*** Mean values for the four remaining animals.

TABLE IX

THE EFFECT OF LIMITING FOOD CONSUMPTION ON SERUM LIPASE (TRIBUTYRINASE) LEVELS OF ADULT MALE RATS

Each food level except the first was fed for one week

(Averages for six animals)

Consumption in gm./day/rat	Serum lipase, units/100 ml.
17± 0.5* (ad lib.)	610± 20*
15	520± 30
12	590± 20
9	670± 20
6	610± 30
3	350± 90**

* Standard error of the mean

** The mean serum tributyrinase value and the standard error of the mean are affected by subnormal levels of the enzyme in the sera of 4 animals (320, 290, 240, and 20 units/100 ml.) which died a few hours after they were bled. The remaining 2 rats, which survived the final bleeding for lipase estimation, had tributyrinase levels within the normal range (700 and 510 units/100 ml.).

TABLE X

THE EFFECT OF ONE WEEK'S POST-DIETARY STARVATION ON THE SERUM TRIBUTYRINASE (UNITS/100 ML. SERUM)(T), AND BODY WEIGHTS (GRAMS)(Δ W) OF ADULT MALE RATS. (MEANS OF SIX ANIMALS).

Diet		Zero Values		Means of Four Weekly Values During Test		Post-Experimental Starvation 1 Week	
		Weight	(T)	(Δ W)	(T)	(Δ W)	(T)
8	8% butter	326 ± 11*	560 ± 20	+43 ± 5	380 ± 10	-66 ± 2	340 ± 40
10	8% linseed oil	319 ± 7	580 ± 20	+24 ± 5	330 ± 10	-68 ± 1	350 ± 20
13	23% butter	334 ± 6	640 ± 10	+45 ± 6	420 ± 10	-67 ± 3	380 ± 30
20	glycerol	310 ± 13	630 ± 20	+37 ± 4	280 ± 10	-65 ± 1	390 ± 20
23	10% Crisco	297 ± 20	660 ± 20	+46 ± 3	350 ± 10	-64 ± 1	390 ± 20
18	fat free	280 ± 9	650 ± 40	+52 ± 7	290 ± 10	-68 ± 1	400 ± 30
19	No A,D, or E.	285 ± 11	630 ± 30	+38 ± 7	290 ± 10	-65 ± 2	420 ± 20
22	stearic acid	279 ± 7	680 ± 20	+31 ± 4	370 ± 20	-61 ± 1	420 ± 20
12	8% olive oil	352 ± 7	610 ± 30	+28 ± 4	290 ± 10	-71 ± 3	420 ± 20
9	8% cottonseed oil	334 ± 9	600 ± 40	+44 ± 5	340 ± 10	-70 ± 1	440 ± 20
11	8% corn oil	321 ± 16	630 ± 20	+34 ± 7	320 ± 10	-64 ± 3	460 ± 10
21	oleic	284 ± 7	650 ± 20	+50 ± 6	440 ± 10	-67 ± 1	490 ± 30

* Standard error of the mean.

Minimum significant difference = ± 40 tributyrinase (Units/100 Ml. Serum).

(VII.) The effects of pre-dietary and post-dietary feeding of Checkers on the serum tributyrinase levels of weanling and adult male rats.

Tables XI and XII illustrate the effects of pre-dietary feeding of Checkers to adult male animals and the post-dietary feeding of Checkers to both adult and weanling male rats. The weanling rats were not fed Checkers prior to the feeding of the test diets because we were primarily interested in noting the effects of the experimental diets immediately after weaning.

It may be noted in Table XI that pre-experimental feeding of Purina Fox Checkers to adult male rats gives rise to relatively high tributyrinase levels and that these can be equalled only by diets containing large amounts of Crisco (diets 6 and 7). The post-dietary feeding of Checkers gives rise to enzyme levels which are somewhat lower than the pre-experimental levels and the difference is statistically significant. This could be accounted for on the basis that it may take longer than one week for the enzyme activity to return to values characteristic of this diet.

The serum tributyrinase levels in weanling rats responded to post-dietary Checker feeding in a manner similar to the adult animals. This may appear surprising in view of the opposing results obtained with the Crisco diets. (Figures 2 and 3). It must be realized, however, that these animals, after seven

weeks on experiment, have reached a period of adolescence and it can no longer be said that the newly established tributyrinase levels are those of "weanling" animals. Even so the picture is puzzling and it is quite possible that other factors may be involved. Once again it can be seen from Tables XI and XII that previous dietary history might influence the serum tributyrinase levels.

TABLE XI

THE EFFECT OF PRE-DIETARY AND POST-DIETARY FEEDING OF CHECKERS ON THE SERUM TRIBUTYRINASE (UNITS/100 ML. SERUM)(T) LEVELS OF ADULT MALE RATS. (MEAN VALUES)

Diet	Checkers 1 week pre-experimental (T)	6 weeks on test diets (T)	Checkers 1 week post-experimental (T)
2	690 ± 20*	360 ± 10*	500 ± 20*
3	630 ± 10	440 ± 10	580 ± 10
4	660 ± 10	410 ± 10	560 ± 10
5	660 ± 20	480 ± 10	630 ± 20
6	770 ± 10	630 ± 10	570 ± 20
7	680 ± 10	550 ± 10	510 ± 40

* Standard error of the mean.

TABLE XII

THE EFFECT OF POST-DIETARY FEEDING OF CHECKERS ON THE SERUM TRIBUTYRINASE (UNITS/100 ML. SERUM)(T) LEVELS OF WEANLING MALE RATS. (MEAN VALUES).

Diet	6 weeks on test diets (T)	Checkers 1 week post-experimental (T)
2	340 ± 10*	420 ± 30*
3	550 ± 10	570 ± 20
4	490 ± 20	550 ± 20
5	480 ± 10	660 ± 20
6	390 ± 20	560 ± 20
7	380 ± 20	420 ± 10

* Standard error of the mean.

(VIII) The effects of various normal and experimental conditions on the serum tributyrinase levels of male rats.

Some odd bits of information were compiled and subjected to statistical analysis with very interesting results. The minimum significant difference for the mean values listed in Table XIII is ±30 tributyrinase units per 100 ml. serum. Tuba and Hoare (36) have reported that the "normal range" for serum tributyrinase levels in adult male rats on a Checker diet is from 460 to 700 units per 100 ml. serum. An examination of Table XIII, with the aid of the minimum significant difference listed above, shows that significant variations due to other factors may occur within this range. Starvation values have been obtained which are well within the reported "normal range" for animals on a Checker diet. Furthermore, it can be seen that variations in the manner in which an experiment is carried out can cause significant differences in the tributyrin splitting power of rat serum. Previous dietary history, for example, can alter the enzyme levels of adult male rats as evidenced by the values for the starvation experiments. In previous sections we have shown that the amount of fat ingested influences the serum tributyrinase levels and once again the most striking effect on the enzyme values (Table XIII) was due to diets free of fat.

It is important to keep in mind, however, that other factors may play an important part in establishing the lipolytic activity of rat serum. The differences in behaviour between adult and weanling rats on Crisco diets have not been fully explained. Other factors which deserve further investigation are, first of all, the relatively high tributyrinase levels established during periods of food deprivation and secondly, the behaviour of this enzyme in weanling rats. Although some light has been thrown on various factors which affect rat serum tributyrinase the priciple problems concerning its exact physiological functions and origin remain unanswered.

TABLE XIII

MEAN TRIBUTYRINASE (UNITS/100 ML. SERUM)(T) LEVELS UNDER VARIOUS NORMAL AND EXPERIMENTAL CONDITIONS

	(T)	Number of Values
Checkers (pre-experimental)	660 ± 10*	148
Checkers (post-experimental) (adult male rats)	530 ± 20	51
Checkers (post-experimental) (weanling male rats)	530 ± 20	52
Prolonged starvation (11 days)	590 ± 10	17
Starvation on a graded Checker diet (4 weeks)	600 ± 20	24
Starvation (1 week post-experimental)	420 ± 10	36
Minimal levels (diets without fat) (diets 18, 19, and 20)	290 ± 10	72

* Standard error of the mean.

Minimum significant difference ± 30.

SUMMARY.

(i) Variations in the serum tributyrinase levels of normal adult and weanling male rats could not be accounted for on the basis of consumption of Purina Fox Checkers. The high tributyrinase levels of alloxan diabetic rats can be correlated to the amount of Checkers ingested daily. In the case of the diabetic rats, however, the amount of food consumed was very much greater and showed more individual variation than in the case of the normal animals.

(ii) Adult male rats showed a positive correlation between the serum tributyrinase levels and the daily consumption of Crisco, a hydrogenated vegetable fat. The opposite effect was obtained with weanling animals. Their serum tributyrinase levels decreased with increased ingestion of Crisco. It is thought that this was possibly due to retarded growth as a result of reduced protein intake on the diets of higher fat content.

(iii) The correlation, in the case of the adult animals, was found to apply not only in the case of dietary Crisco but also with various other fats which were tested. The nature of the dietary fat was also found to influence the enzyme activity. The effects due to the different fats cannot be accounted for on the basis of differences in fat consumption.

(iv) Various derived lipids and fat soluble vitamins were also fed in order to test their effects on the serum tributyrinase levels. It was found that diets free of fat produced minimal levels and ingestion of the fat soluble vitamins A, D, and E, or glycerol, did not alter these basal enzyme levels. Oleic acid and stearic acid both increased the serum tributyrinase levels when ingested by adult male rats and this effect was most pronounced with oleic acid.

(v) Cod liver oil itself affects the serum tributyrinase levels and undoubtedly modifies the effects produced by other dietary fats. Concentrates of the fat soluble vitamins or the use of a mixture of the pure vitamins could replace the cod liver oil usually used in synthetic diets and thereby obviate its effects.

(vi) From inanition experiments it was possible to conclude that a tributyrin hydrolyzing enzyme present in the serum of adult male rats was in some way connected with the utilization of the body depot fats during starvation.

(vii) Information was compiled and subjected to statistical analysis in order to demonstrate differences in the serum tributyrinase levels due to variations in experimental procedure, starvation, and diets deficient in fat. It appears that statistically significant differences may occur within what is usually considered a "normal range" for the enzyme.

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ON RAT SERUM LIPASE

III. THE EFFECT OF TOTAL FOOD CONSUMPTION AND OF DIETARY HYDROGENATED VEGETABLE FAT

By Jules Tuba and Jack D. Taylor

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ON RAT SERUM LIPASE

III. THE EFFECT OF TOTAL FOOD CONSUMPTION AND OF DIETARY HYDROGENATED VEGETABLE FAT[1]

By Jules Tuba and Jack D. Taylor

Abstract

There is no correlation in either weanling or adult normal male rats between daily food consumption of a stock laboratory diet and serum tributyrinase activity. Limiting the food intake of adult male rats has no statistically significant effects on serum tributyrinase. The abnormally elevated serum tributyrinase levels of alloxan diabetic adult male rats may be accounted for on the basis of increased food consumption. Weanling and adult male animals maintained on synthetic diets containing from 5 to 60% fat show a correlation between serum tributyrinase concentration and daily fat consumption. In the case of adult rats increased fat ingestion is accompanied by increased enzyme activity. However, with weanling rats increased fat consumption results in lowered tributyrinase concentrations and retarded growth. The results with growing animals are attributed to the decreased intake of food, and the corresponding decrease in protein consumption, which accompanies the increase in dietary fat concentration. Replacement of the synthetic diets by the stock laboratory diet is followed by altered tributyrinase levels in both weanling and adult animals.

Introduction

A striking characteristic of adult rat serum lipase noted by Tuba and Hoare (5) was that throughout a starvation period of one week enzyme concentrations remained at normal levels. Repetition of this experiment by us indicated that this finding held true for periods of starvation up to 14 days and only a day or so before death did lipase values decrease. It therefore appeared probable that variations in food intake would not likely account for the altered lipase levels which were found associated with various experimental states (5). Independence of serum lipase concentration and daily food consumption would thus be the opposite of findings in this laboratory with rat serum alkaline phosphatase. In the case of the latter enzyme, Tuba and Madsen (6) have shown a highly significant correlation with consumption of food, particularly of fat, except in the presence of certain modifying dietary factors. In spite of the findings with starved animals, an attempt was made to correlate serum lipase levels of both weanling and adult male rats with the daily ingestion of stock laboratory diet and of a hydrogenated vegetable fat, Crisco. The relationship of food consumption and activity of the enzyme was considered in alloxan diabetic adult male rats as well.

Experimental

Animals were housed in individual cages and were given food as described below for individual experiments. Weights were noted at weekly intervals and these observations were used as a check on the health or rate of growth

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of the animals on the different diets. Data concerning weights is included in the tables only when correlation with lipase concentrations has been found.

In order to determine the effect of total daily food consumption the animals were maintained on the stock laboratory diet of Purina Fox Checkers, which were powdered in order to minimize scattering by the rats. This was fed to weanling, normal adult, and alloxan diabetic adult rats. The effect of fat was tested by feeding synthetic diets which contained 5%, 10%, 25%, 40%, and 60% of this dietary constituent to weanling and normal adult male rats. Each synthetic diet contained 2% cod liver oil and the remainder of the fat was made up with Crisco, a hydrogenated vegetable fat. Each of this group of diets contained 20% casein, 40% McCollum's salt mixture, and the remaining fraction was made up with sucrose in every case. Adequate amounts of the following vitamins were added: thiamine hydrochloride, pyridoxine hydrochloride, calcium pantothenate, niacin, riboflavin, and choline. Consumption by each animal was noted daily. In order to simplify reports of data and statistical studies, consumption figures for each group were totaled every week, and the values appearing in the tables are the averages in grams per rat per day for each week of the test period for each diet.

Serum tributyrinase levels were determined once a week by means of the titrimetric micromethod of Tuba and Hoare (4). The tributyrinase activity of serum in units is equivalent to the number of milliliters of 0.025 *N* sodium hydroxide required to neutralize the butyric acid set free by the enzyme contained in 100 ml. serum in 30 min. at pH 8.05 and 37° C.

Results

The Effect of Total Food Consumption

In Normal Weanling and Adult Male Rats

Six weanling male rats, 21 days old, and six adult rats were placed on the diet of powdered Checkers, which they received ad libitum for six weeks. The means of the results for these two experiments are given in Table I. There is no correlation between daily food consumption and serum tributyrinase activity in either growing or mature animals ($r = 0.06$ and 0.01 respectively). There is, however, a highly significant correlation between the weights of the animals and tributyrinase levels in the weanling group ($r = 0.56$ at the 1% level). This further confirms the observations of Tuba and Hoare (5) that serum tributyrinase levels increase from low levels at birth to higher levels which become constant in the mature animal. The lack of correlation between the weights of the adult group and their serum tributyrinase levels ($r = 0.16$) is therefore to be expected.

The effect of limiting the food intake of normal adult animals was tested also. Six mature male rats which had been eating powdered Checkers ad libitum for several weeks were then limited to 15 gm. each daily for one week. During the following four weeks they were maintained on daily levels of 12, 9, 6, and 3 gm. of food for one week on each successive decrement. The mean serum tributyrinase levels for the group remained within normal limits for the

TABLE I

THE RELATIONSHIP BETWEEN DAILY FOOD CONSUMPTION AND SERUM LIPASE (TRIBUTYRINASE) LEVELS IN WEANLING AND ADULT MALE RATS RECEIVING A DIET OF POWDERED ANIMAL CHECKERS

(Averages for six animals in each group)

Time after beginning of experiment, weeks	Weanling rats			Adult rats	
	Consumption in gm./day/rat	Serum lipase, units/100 ml.	Weights in grams	Consumption in gm./day/rat	Serum lipase, units/100 ml.
1	12.0 ± 0.5*	—	73 ± 5*	22.0 ± 0.5*	760 ± 30*
2	15.0 ± 0.5	510 ± 30*	113 ± 6	22.0 ± 0.5	610 ± 40
3	18.0 ± 0.5	610 ± 10	156 ± 7	21.0 ± 0.5	560 ± 20
4	19.0 ± 0.5	590 ± 10	187 ± 5	22.0 ± 0.5	580 ± 10
5	20.5 ± 0.5	600 ± 20	228 ± 6	21.0 ± 0.5	680 ± 30
6	20.0 ± 0.5	610 ± 20	240 ± 5	20.0 ± 0.5	660 ± 40

* *Standard error of the mean.*

first four weeks of the experiment. Only at the end of the week on a daily intake of 3 gm. of food did the results given in Table II show a decline from normal mean serum lipase concentrations. Once more there is no significant

TABLE II

THE EFFECT OF LIMITING FOOD CONSUMPTION ON SERUM LIPASE (TRIBUTYRINASE) LEVELS OF ADULT MALE RATS

Each food level except the first was fed for one week
(Averages for six animals)

Consumption in gm./day/rat	Serum lipase, units/100 ml.
17 ± 0.5* (ad libitum)	610 ± 20*
15	520 ± 30
12	590 ± 20
9	670 ± 20
6	610 ± 30
3	350 ± 90**

* *Standard error of the mean.*

** *The mean serum tributyrinase value and the standard error of the mean are affected by subnormal levels of the enzyme in the sera of four animals (320, 290, 240, and 20 units per 100 ml.) which died a few hours after they were bled. The remaining two rats, which survived the final bleeding for lipase estimation, had tributyrinase levels within the normal range (700 and 510 units per 100 ml.).*

correlation between food consumption and serum tributyrinase levels. The value of r, which is at the extreme lower limit of the 5% level of significance, is 0.33. Weight and activity of the enzyme showed no correlation in this experiment.

In Alloxan Diabetic Adult Male Rats

Six alloxan diabetic rats with pronounced hyperglycemia were used for this experiment. The mean serum lipase activity of the group was well above normal, in accordance with the findings of Tuba and Hoare (5). These animals were fed powdered Checkers ad libitum for six days. The amount of food eaten by each animal was measured daily and serum lipase activity was estimated every two days. The mean lipase values for this group are given in Table III as well as the mean average food consumption for the 24 hr. immediately preceding the bleeding for determination of tributyrinase levels.

TABLE III

THE RELATIONSHIP BETWEEN DAILY FOOD CONSUMPTION AND SERUM LIPASE (TRIBUTYRINASE) LEVELS IN ALLOXAN DIABETIC ADULT MALE RATS

(Averages are for six animals in the group)

Time after beginning of experiment, days	Consumption in gm./day/rat	Serum lipase in units/100 ml.
2	32 ± 1.3*	800 ± 60*
4	35 ± 1.2	890 ± 100
6	33 ± 0.9	810 ± 100

* *Standard error of the mean.*

The regression line shown in Fig. 1 was calculated from the three lipase values and the six daily consumption levels determined for each rat during the course of the above experiment. The calculated equation for this regression line is $T = 44C - 636$ (T = tributyrinase and C = food consumption) and this has a highly significant t value = 3.1 ($P < 0.01$). This, together with the value of $r = 0.64$ for the data of Table III, indicates that there is a highly significant correlation between food ingested and serum tributyrinase concentration in alloxan diabetic adult male rats.

A simple arithmetic comparison of the consumption and enzyme data of the preceding three tables seems to indicate that the abnormally high tributyrinase activity associated with alloxan diabetes in rats is largely attributable to the increase in food consumption. This can readily be proved statistically. We have used the individual consumption and lipase data for six alloxan diabetic rats (Table III) and for six normal adult rats (last three weeks in Table I) and there is a highly significant correlation; $r = 0.63$ with a t value of 3.59 at the 1% level.

It was shown above that this correlation does not exist in normal adult rats receiving food ad libitum or in limited amounts. When the animals are starved or receive restricted amounts of food it would appear that the levels of serum tributyrinase must remain at or above certain unknown values and that the rats die when the enzyme concentration falls below these levels. The lack of correlation in normal adult rats eating unrestricted amounts of

powdered Checkers is probably due to the wide fluctuations in the activity of the enzyme in a group of animals eating much the same quantity of food from day to day and week to week (Table I), and it should be noted that the value

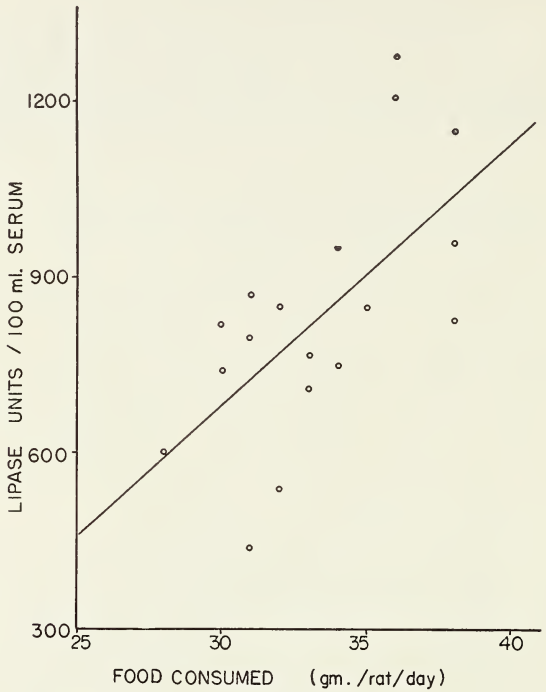

FIG. 1. The regression line for the relationship between daily consumption of a stock laboratory diet and serum tributyrinase levels in alloxan diabetic adult male rats.

of r for this experiment is almost significant. Only when both consumption and lipase vary markedly from the normal ranges, as in the alloxan diabetic group (Table III) is this correlation readily shown.

The fact that a correlation between food intake and serum tributyrinase concentration could be obtained under certain conditions led to a consideration of the effect on the enzyme of various dietary constituents, notably fat. In this regard, Hess and Viollier (3) have reported that the absence of fat from the diet reduces the serum tributyrinase content of rat plasma.

The Effect of Dietary Hydrogenated Vegetable Fat

In Weanling Male Rats

Groups of 10 weanling male rats were fed ad libitum for six week periods on a number of synthetic diets containing the various levels of fat indicated in Table IV. Each diet contained 2% cod liver oil and the balance of the fat

TABLE IV

THE EFFECT OF DIETARY FAT CONCENTRATION ON MEAN DAILY FOOD CONSUMPTION (GM. PER DAY) (C), MEAN DAILY FAT CONSUMPTION (GM. PER DAY) (F), AND SERUM TRIBUTYRINASE (UNITS PER 100 ML.) (T) OF WEANLING MALE RATS

(Averages are for 10 animals in each group)

Fat in diet, %	2 weeks			3 weeks			4 weeks			5 weeks			6 weeks		
	C	F	T	C	F	T	C	F	T	C	F	T	C	F	T
5	11.0	0.55	530	10.8	0.54	550	11.4	0.57	540	14.1	0.70	570	14.1	0.70	530
10	8.2	0.82	550	9.1	0.91	500	12.0	1.20	470	12.3	1.23	470	10.0	1.00	470
25	10.2	2.53	510	10.2	2.53	480	13.5	3.38	530	10.2	2.53	450	12.0	3.00	410
40	6.0	2.40	380	7.8	3.12	440	7.5	3.00	370	12.4	4.97	450	14.0	5.52	330
60	6.1	3.96	400	7.0	4.20	430	8.3	4.98	370	7.6	4.56	400	6.2	3.72	300

was made up with Crisco. Lipases were estimated weekly except at the end of the first week, when the animals were too small to bleed. The daily food intake of individual animals was variable on some of the diets, especially when higher concentrations of fat were fed. Consequently the values for food consumption reported in Table IV in grams eaten per rat per day are the means of the total food eaten by the 10 animals of each group over a period of one week. From the food consumption data the amount of fat eaten per rat per day has been calculated.

A significant correlation exists between the amount of fat eaten daily by weanling rats and the levels of serum tributyrinase. The value of $r = 0.71$. The formula of the regression line shown in Fig. 2 is $T = -33F + 540$ (T = tributyrinase and F = fat consumption) and this has a highly significant value of $t = 5.2$ at the 1% level. The standard error of estimate for the line in $\pm$ 51.

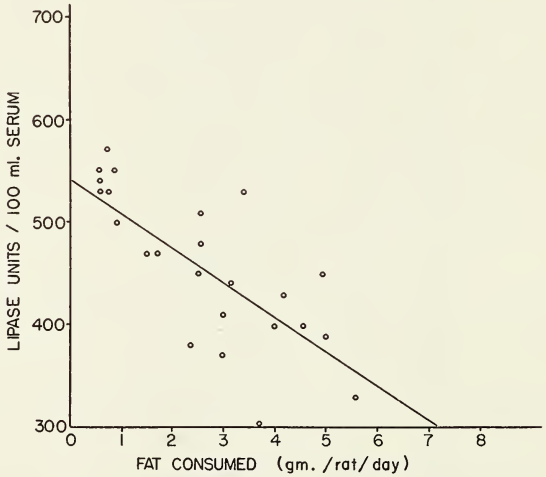

FIG. 2. The regression line for the relationship between serum tributyrinase levels and fat consumed per day by normal weanling rats.

In Adult Male Rats

The preceding experiment was repeated with a group of 10 normal adult male rats. Lipases were determined at the end of each week of the feeding period. As with the weanling animals the average daily consumption of food became less as the fat concentration of the diets was increased. The daily consumption of fat once more was higher as the dietary fat concentration was increased. A highly significant correlation exists between the consumption and enzyme data given in Table V ($r = 0.78$ at the 1% level).

TABLE V

THE EFFECT OF DIETARY FAT CONCENTRATION ON MEAN DAILY FOOD CONSUMPTION (GM. PER DAY) (C), MEAN DAILY FAT CONSUMPTION (GM. PER DAY) (F), AND SERUM TRIBUTYRINASE (UNITS PER 100 ML.) (T) OF ADULT MALE RATS

(Averages are for 10 animals in each group)

Fat in diet, %	1 week			2 weeks			3 weeks			4 weeks			5 weeks			6 weeks		
	C	F	T	C	F	T	C	F	T	C	F	T	C	F	T	C	F	T
5	14.1	0.71	400	13.6	0.68	410	11.4	0.57	440	12.0	0.60	470	14.6	0.73	420	15.0	0.75	450
10	14.7	1.47	410	13.0	1.30	370	12.0	1.20	470	15.0	1.50	420	14.8	1.48	400	14.6	1.46	380
25	15.2	3.80	520	12.6	3.15	520	11.8	2.95	540	11.4	2.85	430	11.3	2.82	440	11.2	2.80	440
40	11.4	4.56	570	11.8	4.72	650	8.8	3.52	660	11.8	4.72	590	12.3	4.92	640	12.0	4.80	660
60	13.6	8.16	590	9.1	5.45	590	8.4	5.04	550	10.4	6.25	500	9.5	5.70	540	9.7	5.83	550

The formula of the regression line shown in Fig. 3 is T = + 33F + 385 (T = tributyrinase and F = fat consumption) and this has a highly significant t value of 6.9 at the 1% level. The standard error of estimate for this regression line is ± 55.

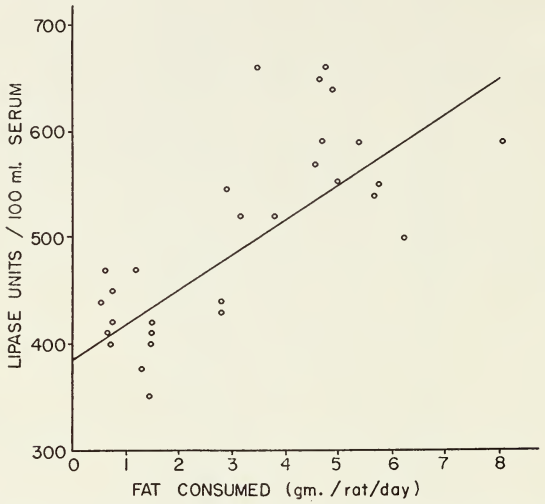

FIG. 3. The regression line for the relationship between serum tributyrinase levels and fat consumed per day by normal adult rats.

A striking fact concerning the regression lines for the above two experiments is that the slopes have opposite signs. The increased lipolytic activity of serum associated with increased fat ingestion in the case of adult animals could be expected if serum tributyrinase has a role in the metabolism of fats. The findings with weanling animals is surprising. The data in Table VI offers some explanation.

TABLE VI

THE RELATIONSHIP BETWEEN AVERAGE GROWTH RATE OF WEANLING MALE RATS (IN GM.) ON VARIOUS DIETARY CONCENTRATIONS OF FAT AND AVERAGE SERUM TRIBUTYRINASE ACTIVITY (T) (UNITS PER 100 ML.)

Diet	Weight after 6 weeks on diet	Weight gain in 6 weeks	T at end of 6 weeks	Weight gain after one week on Checkers	T after one week on Checkers	Change in T produced by Checkers
Checkers, 5% fat	240	185	610	30	—	—
Synthetic, 5% "	213	142	570	40	610	+ 40
" 10% "	201	149	470	7	550	+ 80
" 25% "	180	121	410	31	660	+250
" 40% "	183	138	330	30	560	+230
" 60% "	147	92	300	41	420	+120

The growth of weanling rats fed the various diets listed in Table VI is best on Checkers, which contain about 5% fat. Growth on the synthetic diets containing 5 and 10% fat is not quite as good, but they are not as palatable as the Checkers, judging by consumption data. The diminished rate of growth on all the synthetic diets can be accounted for on the basis of lowered daily food consumption which in nine-week-old rats (at the end of the six week feeding periods) ranges from 20 gm. on the Checker diet to 6.2 gm. on the 60% fat diet. The synthetic diets which contain more than 5% fat not only interfere with the rate of growth, because of inadequate protein intake, but tend to maintain the mean lipase levels near the values of 317–484 units per 100 ml. reported by Tuba and Hoare (5) for three- to four-week-old rats.

After the termination of the feeding experiments, each group of animals was placed on Checkers for one week. An improvement in the rate of growth was noted in all but one group, and the enzyme levels increased toward the range of values characteristic of the stock laboratory diet.

Deuel and his co-workers (2) found that rats fed ad libitum on synthetic diets containing 5 to 50% fat showed better growth than those fed lower concentrations of fat or a stock laboratory diet. Their results were similar for cottonseed oil and a hydrogenated vegetable fat, margarine. Barki, Collins, Elvehjem, and Hart (1) fed various levels of three vegetable oils and butter to growing rats and observed that the dietary concentration of each for optimal growth was characteristic of the fat. These levels were 10% for corn oil and 35% for butter fat, whereas coconut oil or soybean oil concentrations had no effect on growth. In our experiments, which were primarily designed for lipase studies, the closest approximation to both "normal" growth and serum tributyrinase activity was obtained with the synthetic diet containing 5% fat. Our results are probably affected to some extent by the fact that each synthetic diet contained 2% cod liver oil. However, experiments are now in progress with this and a number of other fats to see what effect they have on growth and lipase concentration.

The adult animals used in our six week feeding tests gained 110 gm. on Checkers and from 85 to 125 gm. on the various synthetic diets. Transference from the synthetic diets to a diet of powdered Checkers for one week resulted in a return of tributyrinase activity to levels ranging from 510 to 630 units per 100 ml. which are typical for the stock laboratory diet.

The problem of constancy of tributyrinase levels during starvation remains unanswered. Since diet does affect the activity of the enzyme, one can only assume until further work is done, that the starvation levels are associated with utilization of body stores of fat.

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